

# **A Complete Microbiomics Solution**

from Collection to Conclusion



### **Standardizing Microbiomics**



#### **Optimizing Microbiomics Workflows**

Advances in DNA sequencing and other genomeenabled technologies have lowered the cost and time requirements needed to sequence any organism. Next-Generation Sequencing of microbial communities has supercharged our ability to research and explore both human and environmental microbial ecosystems. This advancement in technology has allowed, for the first time, large-scale, multi-lab studies of microbial communites<sup>1</sup>.

Early quality control studies of microbiomic research suggest that this vast new frontier of research is littered with potential sources for error and bias. From collection to sequencing, the potential for variation at each step in the microbiomic workflow is enormous.

During the collection and transportation phase, depending on the quality of the method, microbes can continue to grow and decay. Nucleic acids can also degrade during this phase. These two factors can lead to overall misrepresentation of the original community profile, causing downstream bias. In the extraction phase, inferior forms of lysis can fail to extract DNA from tough-to-lyse microbes (e.g. Gram-positive bacteria and yeast), leading to the underrepresentation of toughto-lyse microbes and overrepresentation of easy-tolyse microbes (e.g. Gram-negative bacteria). During sequencing, different library preparation methods can cause biased coverage depending on the GC content of the organisms.

As multi-lab, longitudinal microbiomic studies become more common, there is an urgent need for standards to establish validated methods for reproducible data<sup>2</sup>.

At Zymo Research, we have made it our goal to eliminate bias across the entire microbiomic workflow. Our new product line, ZymoBIOMICS<sup>®</sup>, is a complete workflow, from collection to analysis, which offers streamlined collection, purification, and the first microbial community standards. Zymo Research has endeavored to develop standards for the advancement and optimization of microbiomic workflows to allow researchers to have increased confidence in their microbiomics data. ZymoBIOMICS<sup>®</sup> Microbial Community Standards are groundbreaking community standards, containing a well-defined and characterized community of Gram-positive bacteria, Gram-negative bacteria and yeast. They are ideal for validating and optimizing microbiomics and metagenomic workflows from extraction to analysis.

At collection, DNA/RNA Shield<sup>®</sup> stabilizes nucleic acids and inactivates all organisms — including pathogens — in your sample, allowing for accurate community profiling. There is no degradation of nucleic acids, creating a perfect molecular snapshot of your sample at the time of collection.

Our ZymoBIOMICS® DNA Mini Kit ensures accurate community profiling by enabling total lysis from any sample, including feces, soil, water, biofilms, swabs, body fluid, etc. DNA extracted from this kit is ultra-pure, inhibitor-free, and ready for all downstream applications, including PCR 16S rRNA gene sequencing and shotgun sequencing.

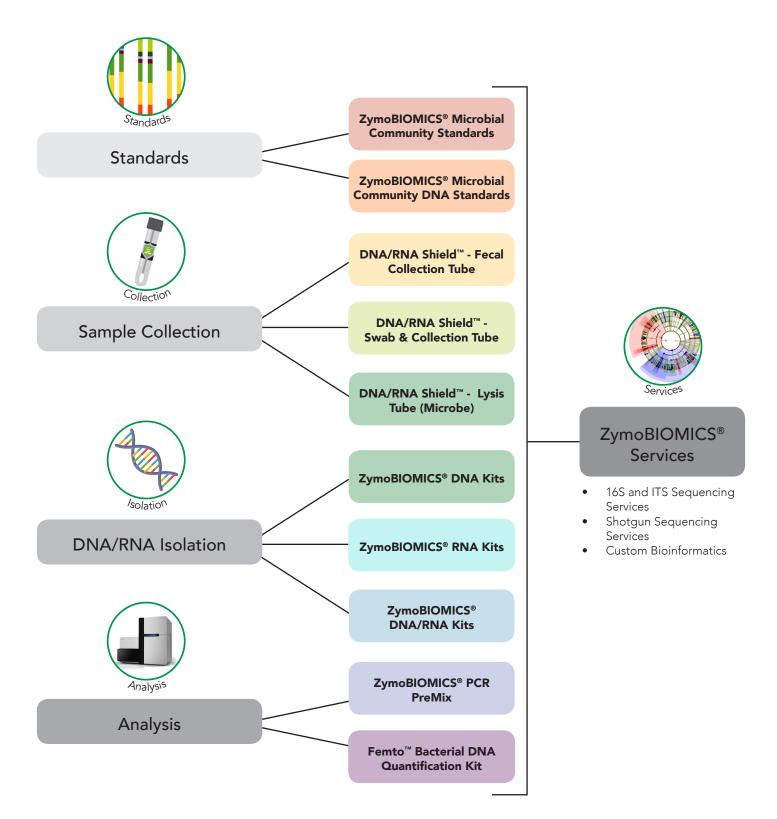
If you would prefer to leave the work to us, we offer a comprehensive list of ZymoBIOMICS<sup>®</sup> Services covering all steps of a microbiomics workflow from collection to analysis. Simply send us your sample and we will handle the rest. All workflows are validated to be non-biased using the ZymoBIOMICS<sup>®</sup> Microbial Community Standards and provide publication-ready data.

ZymoBIOMICS® - Standardizing Microbiomics

#### Learn more at www.zymoresearch.com/zymobiomics-portfolio

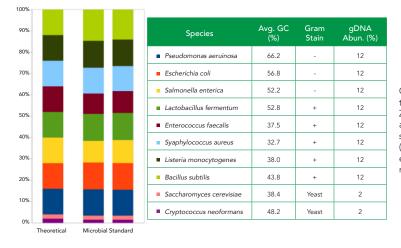
<sup>1.</sup> Sinha, Rashmi, et al. "The microbiome quality control project: baseline study design and future directions." Genome biology 16.1 (2015) 2. Stulberg, Elizabeth, et al. "An assessment of US microbiome research. "Nature Microbiology 1 (2016): 15015.

## A Complete Microbiomics Solution from Collection to Conclusion



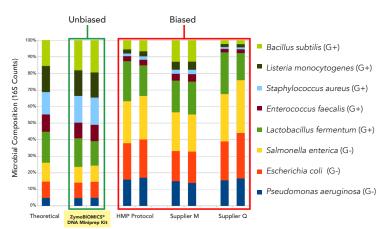
### ZymoBIOMICS® Microbial Community Standard

- Mock microbial community of well-defined composition.
- Ideal for the validation, optimization, and quality control of microbiomics and metagenomic workflows.
- Perfect for assessing bias of DNA extraction methods since it contains both tough and easy-to-lyse microbes.



#### Accurate Characterization

Containing three easy-to-lyse Gram-negative bacteria, five toughto-lyse Gram-positive bacteria, and two tough-to-lyse yeasts, the ZymoBIOMICS® Microbial Community Standard is perfect for assessing bias in various DNA extraction methods. The microbial standards are accurately characterized, with a wide GC range (15%-85%) and contain negligible impurities (<0.01%), enabling easy exposure of artifacts, errors, and bias in microbiomics or metagenomic workflows.



#### Find Your Bias & Eliminate It

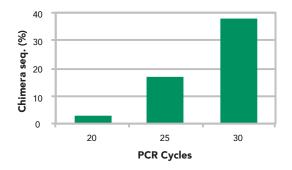
ZymoBIOMICS  $^{\odot}$  Microbial Community Standard was used to compare different DNA extraction protocols. DNA samples were profiled by 16S rRNA gene targeted sequencing.

| Product                                   | Cat. No. | Size     |
|---|----------|----------|
| ZymoBIOMICS® Microbial Community Standard | D6300    | 10 preps |

### ZymoBIOMICS® Microbial Community DNA Standard

- A DNA standard of well-defined composition.
- Ideal for the validation, optimization, and quality control of microbiomics and metagenomics workflows.
- The DNA has a wide GC range of 15% 85%.

Address & Reduce PCR Chimera

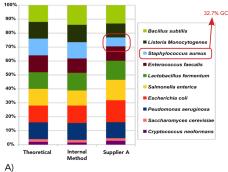


PCR chimera increase the number of PCR cycles during the library preparation process of 16S rRNA gene targeted sequencing. 20 ng ZymoBIOMICS® Microbial Community Standard was used a template. The PCR was performed with ZymoBIOMICS® Taq PreMix master mix and with primers that target the v3-4 region of 16S rRNA gene. Chimera percentage was determined with Uchime and using the 16S rRNA genes of the 8 bacterial strains in the standard as reference.

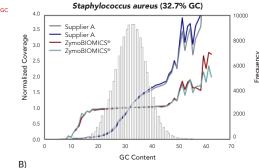
#### Avg. GC (%) Gram gDNA Abun. (% 1009 Species 90% Pseudomonas aeruinosa 66.2 12 80% Escherichia coli 56.8 12 70% Salmonella enterica 52.2 12 60% Lactobacillus fermentum 52.8 + 12 509 Enterococcus faecalis 37 5 + 12 40% Syaphylococcus aureus 32.7 + 12 30% Listeria monocytogenes 38.0 + 12 20% + 12 Bacillus subtilis 43.8 10% Saccharomyces cerevisiae 38.4 2 Yeast 0% Theoretical DNA Standard Cryptococcus neoformans 48.2 Yeast 2

Accurate Characterization

DNA from Gram-negative bacteria, five Gram-positive bacteria, and two tough-tolyse yeasts. The ZymoBIOMICS<sup>®</sup> Microbial Community Standards are perfect for assessing bias in popular extraction methods. The microbial standards are accurately characterized, with a wide GC range (15%-85%) and contain negligible impurities (<0.01%), enabling easy exposure of artifacts, errors, and bias in microbiomics or metagenomic workflows.



#### Assess GC Bias & Eliminate It

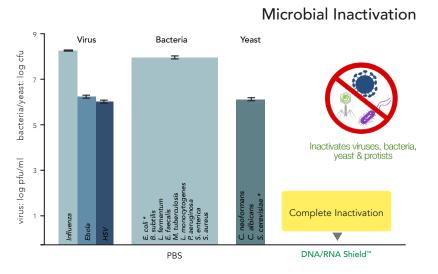


A) Assessing bias of two different library preparation processes in shotgun metagenomic sequencing using ZymoBIOMICS® Microbial Community DNA Standard. Compared to our ZymoBIOMICS® Services, the Supplier A kit has some bias due to GC content variation. Sequencing was performed on MiSeq (2 x 150 bp). B) Raw reads were mapped to the 10 microbial genomes to evaluate the potential effect of GC content on sequencing coverage. Normalized coverage was calculated by normalization with the average sequencing coverage of each genome.

| Product                              | Cat. No. | Size     |
|--------------------------------------|----------|----------|
| ZymoBIOMICS® Microbial Community DNA | D6305    | 200 ng   |
| Standard                             | D6306    | 2,000 ng |

### **DNA/RNA Shield<sup>™</sup> Collection Devices**

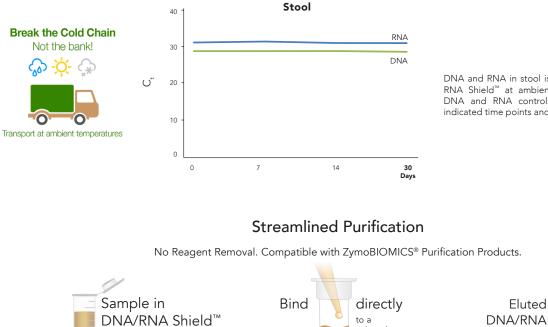
Take a molecular snapshot of your sample with DNA/RNA Shield<sup>™</sup>. This stabilization reagent breaks the cold chain and ensures nucleic acid stability during sample storage/transport at ambient temperatures. DNA/RNA Shield<sup>™</sup> effectively lyses cells and inactivates nucleases and infectious agents, and it is compatible with various collection and storage devices (vacuum tubes, swabs (nasal, buccal, fecal), etc.).



Viruses, bacteria and yeast are effectively inactivated by DNA/RNA Shield<sup>™</sup>. Samples containing the infectious agent (virus, bacteria, yeast) were treated for 5 minutes with DNA/RNA Shield™ or mock (PBS). Titer (PFU) was subsequently determined by plaque assay. Validated by: Influenza A - D. Poole and Prof. A. Mehle, Department of Medical Microbiology and Immunology, University of Wisconsin, Madison; Ebola (Kikwit) - L. Avena and Dr. A. Griffiths, Department of Virology and Immunology, Texas Biomedical Research Institute; HSV-1/2 - H. Oh, F. Diaz and Prof. D. Knipe, Virology Program, Harvard Medical School; E. coli, L. fermentum, B. subtilis, S. cerevisiae - Zymo Research Corporation).

\*Disclaimer: This graph only displays results from E. coli inactivation. Each microbe was tested independently and were combined into one graph for brevity. Bacterial cultures were grown between 108 - 109 cells and yeast cultures were grown between 107 - 108 cells.

#### Nucleic Acid Stabilization at Ambient Temperature



Simply add

binding reagent

DNA and RNA in stool is effectively stabilized in DNA/ RNA Shield<sup>™</sup> at ambient temperature. Graphs show: DNA and RNA controls from stool purified at the indicated time points and analyzed by (RT)qPCR.

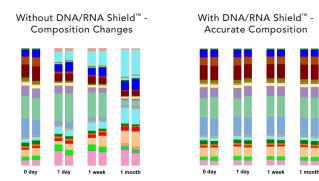
ready to use

spin-column o

. MagBeads

- Provides an accurate "molecular snapshot" of the sample at the time of collection by preserving nucleic acids at ambient temperature and inactivating microbes.
- Nucleic acid preservation (at ambient temperature; cold-free).
- Pathogen inactivation (bacteria, fungus, parasites & viruses).
- Streamlined purification (no reagent removal, universally compatible, automatable).

#### DNA/RNA Shield<sup>™</sup> Preserves Microbial Composition at Ambient Temperature



Microbial composition of stool is unchanged after one month at ambient temperature with DNA/RNA Shield<sup>™</sup>. Stool samples suspended in DNA/RNA Shield<sup>™</sup> and stored at room temperature were compared to stool without preservative for one month. They were sampled at the indicated time points and processed with ZymoBIOMICS<sup>®</sup> DNA Mini Kit. The extracted DNA was then subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Samples stored with DNA/RNA Shield<sup>™</sup> had a constant microbial composition while the samples stored without shifted dramatically.

#### Available DNA/RNA Shield<sup>™</sup> Devices for Microbial Studies



DNA/RNA Shield<sup>™</sup> - Fecal Collection Tube (with scoop) DNA/RNA Shield<sup>™</sup> - Swab & Collection Tube

ritan® DNA/RNA Shield<sup>Tw</sup>



DNA/RNA Shield<sup>™</sup> - Lysis Tube (Microbe)

| Product  | Cat. No.                         | Size   |
|--|----------------------------------|--|
| DNA/RNA Shield <sup>™</sup> - Lysis Tube (Microbe)           | R1103                            | 50 pack  |
| DNA/RNA Shield <sup>™</sup> - Lysis Tube (Microbe) with Swab | R1104                            | 50 tubes/50 swabs  |
| DNA/RNA Shield <sup>™</sup> - Swab & Collection Tube         | R1106<br>R1107<br>R1108<br>R1109 | 10 pack (1 ml fill)<br>50 pack (1 ml fill)<br>10 pack (2 ml fill)<br>50 pack (2 ml fill) |
| DNA/RNA Shield™ - Fecal Collection Tube                      | R1101                            | 10 pack  |

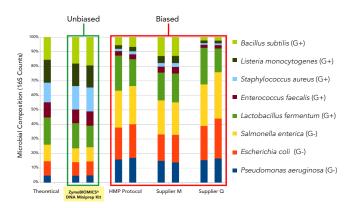
## ZymoBIOMICS® DNA Kits

Streamlined Workflow

- Ultra-pure, inhibitor-free DNA from many microbiomic sample types (feces, soil, water, biofilms, swabs, body fluid, etc.) that is ideal for all downstream applications including PCR, arrays, 16S rRNA gene sequencing, and shotgun sequencing.
- Innovative lysis system enables efficient and unbiased lysis of microbes including Gram-positive/negative bacteria, fungus, protozoans, and algae for accurate microbial community profiling.



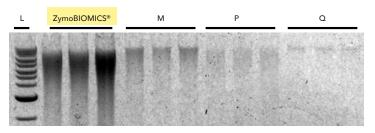
Learn more and view additional formats at www.zymoresearch.com/zymobiomics-portfolio



#### Accurate Community Profiling

The ZymoBIOMICS® DNA Miniprep Kit provides accurate representation of the organisms extracted from the ZymoBIOMICS® Microbial Community Standard.

#### **Superior Yields**

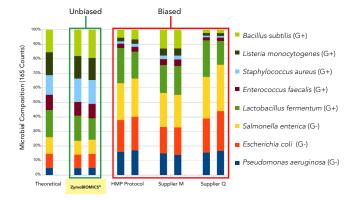


The ZymoBIOMICS® DNA Miniprep Kit provides superior yields when compared to Suppliers M, P, and Q.

| Product   | Cat. No.        | Size                 |
|---|-----------------|----------------------|
| ZymoBIOMICS® DNA Miniprep Kit   | D4300<br>D4300T | 50 preps<br>10 preps |
| ZymoBIOMICS® DNA Miniprep Kit<br>(Lysis Matrix Not Included)                              | D4304           | 50 preps             |
| ZymoBIOMICS <sup>®</sup> DNA Microprep Kit  | D4301           | 50 preps             |
| ZymoBIOMICS® 96 DNA Kit<br>(includes ZR BashingBead <sup>™</sup> Lysis Rack)              | D4303           | 2 x 96 preps         |
| ZymoBIOMICS <sup>®</sup> 96 DNA Kit<br>(includes ZR BashingBead <sup>™</sup> Lysis Tubes) | D4309           | 2 x 96 preps         |
| ZymoBIOMICS® 96 DNA Kit<br>(Lysis Matrix Not Included)                                    | D4307           | 2 x 96 preps         |

### ZymoBIOMICS® 96 Magbead DNA Kit

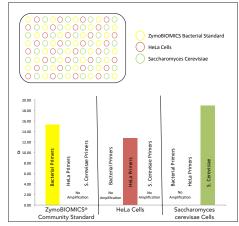
- High-throughput purification of high quality, inhibitor-free DNA from any sample including feces, soil, water, biofilms, swabs, saliva, and body fluids.
- The ZymoBIOMICS<sup>®</sup> innovative lysis system enables efficient and unbiased lysis of microbes including Grampositive/negative bacteria, fungi, protozoans, algae, etc.
- The automation friendly workflow enables samples to be processed in as little as 90 minutes for 96 preps.



#### Accurate Profiling

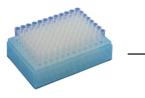
The ZymoBIOMICS® 96 MagBead Kit provides accurate representation of the organisms extracted from the ZymoBIOMICS® Microbial Community Standard.

### No Cross-Contamination



The ZymoBIOMICS<sup>®</sup> 96 MagBead DNA Kit provides cross-contamination free samples across a standard 96-well plate purification performed on a liquid handler. Samples were evaluated using quantitative PCR with primer sets targeted at the bacterial 16S gene, the human LINE gene, and the fungal ITS gene. PCR was performed in technical duplicates.

### No Precipitation or Centrifugation Required



Bias-free Lysis



Quick Bind, Wash, Elute Workflow



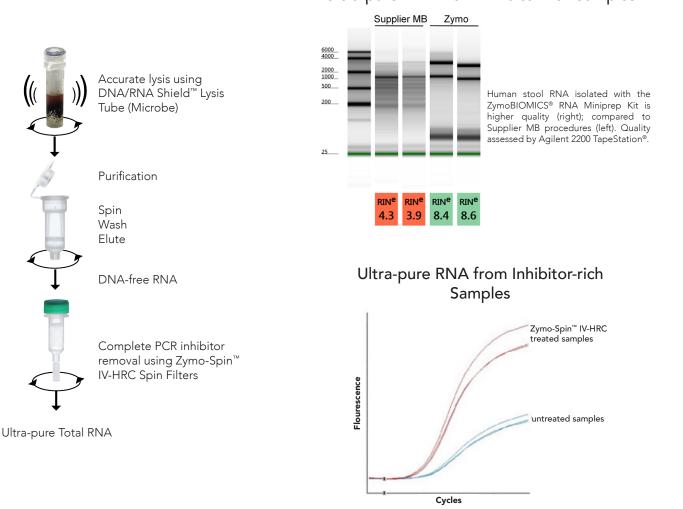
Superior Yields and Integrity Ultra-Pure DNA

| Product  | Cat. No. | Size         |
|--|----------|--------------|
| ZymoBIOMICS <sup>®</sup> 96 MagBead DNA Kit (includes<br>ZR BashingBead™ Lysis Rack) | D4302    | 2 x 96 preps |
| ZymoBIOMICS® 96 MagBead DNA Kit (Lysis<br>Matrix Not Included)                       | D4306    | 2 x 96 preps |
| ZymoBIOMICS® 96 MagBead DNA Kit (includes<br>ZR BashingBead™ Lysis Tubes)            | D4308    | 2 x 96 preps |

# ZymoBIOMICS® RNA Kits

Streamlined Workflow

- Rapid, robust, and simple purification of high quality, inhibitor-free total RNA (including small/micro RNAs) from any sample including feces, soil, water, biofilms, swabs, saliva, and body fluids, etc.
- ZymoBIOMICS<sup>®</sup> innovative lysis system enables efficient and unbiased lysis of microbes including Gram-positive/ negative bacteria, fungus, protozoans, algae, viruses, etc.
- DNA-free RNA is ready for use in any downstream application. DNase I included.



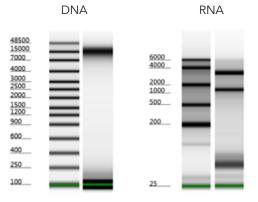
### Ultra-pure RNA from Inhibitor-rich Samples

Total RNA isolated from human stool with or without inclusion of the Zymo-Spin™ IV-HRC Spin Filter during the ZymoBIOMICS® RNA Miniprep Kit protocol. Earlier amplification cycles indicate complete removal of PCR inhibitors.

| Product                       | Cat. No. | Size     |
|-------------------------------|----------|----------|
| ZymoBIOMICS® RNA Miniprep Kit | R2001    | 50 preps |

### ZymoBIOMICS® DNA/RNA Miniprep Kit

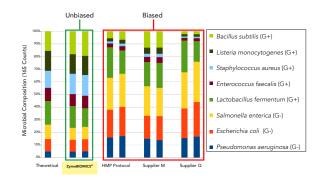
- Rapid, robust, and simple purification of high quality, inhibitor-free DNA and total RNA (including small/micro RNAs) from any sample including feces, soil, plant, water, biofilms, swabs, saliva, body fluids, etc.
- ZymoBIOMICS<sup>®</sup> innovative lysis system enables efficient and unbiased lysis of microbes including Gram-positive/ negative bacteria, fungus, protozoans, algae, viruses, etc.
- High-quality DNA and DNA-free RNA is ready for use in any downstream application. DNase I included.



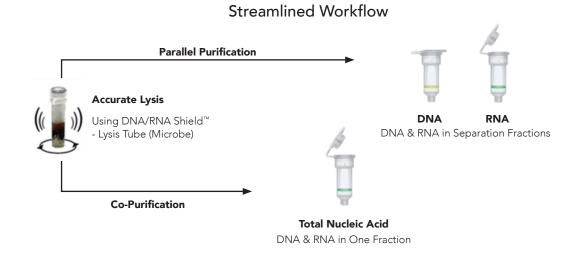
**Superior Yields** 

Human stool genomic DNA and total RNA isolated with the ZymoBIOMICS® DNA/RNA Miniprep Kit is highly intact. Quality assessed by Agilent 2200 TapeStation®.

#### Accurate Community Profiling



The ZymoBIOMICS® DNA/RNA Miniprep Kit provides accurate representation of the organisms extracted from the ZymoBIOMICS® Microbial Community Standard.

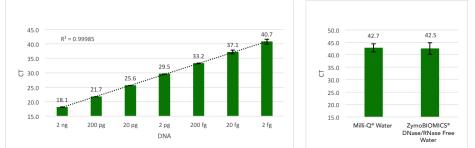


| Product                           | Cat. No. | Size     |
|-----------------------------------|----------|----------|
| ZymoBIOMICS® DNA/RNA Miniprep Kit | R2002    | 50 preps |

## ZymoBIOMICS® PCR PreMix

- Simply add water, DNA, primers and go!
- Certified low-bioburden.
- Robust amplification for the detection of low copy DNA.
- Ideal for highly sensitive applications.

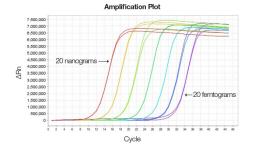
#### Sensitive Detection Range Free of Bacterial DNA



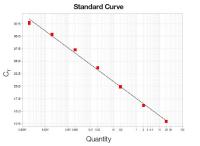
Quantification of no template controls (NTCs) via realtime PCR was determined by amplification of the 16S rRNA gene, after the addition of 2.5  $\mu$ M SYTO® 9 to a 20  $\mu$ I reaction volume. Real-time PCR was performed for 45 cycles to determine the amount of bacterial contamination. NTCs include Millipore filtered water and DEPC treated Millipore filtered water.

### Femto<sup>™</sup> Bacterial DNA Quantification Kit

- Quantify as little 20 femtograms of DNA in as little as 1  $\mu$ l of sample.
- High specificity and sensitivity for DNA in a background of non-target DNA.
- Fast and simple: add samples to the PreMix... and quantify.



#### **Reliable Quantification**



Reliable standards for the qualification of bacterial DNA: Bacterial DNA Standards (measured in dupliactes) comprise a 10-fold dilution series ranging from 20 ng to 20 fg.

| Product   | Cat. No.       | Size                |
|---|----------------|---------------------|
| ZymoBIOMICS® PCR Premix                             | E2056<br>E2057 | 50 rxns<br>200 rxns |
| Femto <sup>™</sup> Bacterial DNA Quantification Kit | E2006          | 100 rxns            |

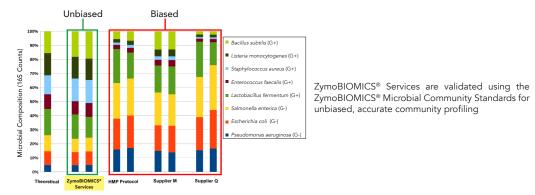
## **ZymoBIOMICS®** Services

- Zymo Research offers the most comprehensive services for 16S rRNA and Shotgun sequencing from any sample type.
- ZymoBIOMICS<sup>®</sup> Services are validated using the ZymoBIOMICS<sup>®</sup> Community Standards to ensure accurate, publication-guality data.
- Services include low-bioburden processing and DNA/RNA isolation using the ZymoBIOMICS<sup>®</sup> product line for the most accurate taxonomic profiling.

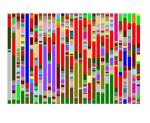
#### A Comprehensive Solution for Microbiomics and Metagenomics



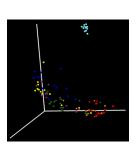
### Validated, Accurate Workflows from Collection to Analysis



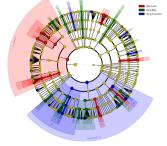
### Comprehensive, Customizable Bioinformatics & Data Analysis



**Composition Barplots** 



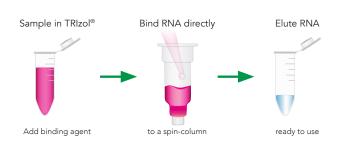




LEfSe Cladogram

### **Related Products**

### Direct-zol<sup>™</sup> RNA Kits

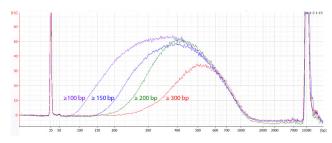


# From TRIzol® to high-quality RNA in 7 minutes without phase separation

| Product                                       | Size                         | Cat. No.                       |
|---|------------------------------|--------------------------------|
| Direct-zol <sup>™</sup> RNA Miniprep Plus     | 50 preps<br>200 preps        | R2070; R2071*<br>R2072; R2073* |
| Direct-zol <sup>™</sup> RNA Miniprep          | 50 preps<br>200 preps        | R2050; R2051*<br>R2052; R2053* |
| Direct-zol <sup>™</sup> RNA Microprep         | 50 preps<br>200 preps        | R2060; R2061*<br>R2062; R2063* |
| Direct-zol <sup>™</sup> -96 RNA Miniprep Plus | 2 x 96 preps<br>4 x 96 preps | R2054; R2055*<br>R2056; R2057* |

\*Supplied with TRI Reagent®

### Select-a-Size DNA Clean & Concentrator® Kits

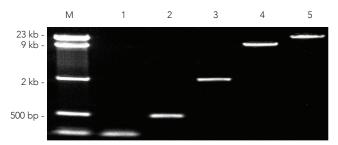


Select-a-Size DNA Clean and Concentrator<sup>®</sup> allows for selection at ≥300 bp, ≥200 bp, ≥150 bp, ≥100 bp and ≥50 bp.

Select for DNA species  $\geq$  50 bp,  $\geq$  150 bp,  $\geq$  200 bp,  $\geq$  300 bp,  $\geq$  700 bp or perform double size selection

| Product                                 | Size     | Cat. No. |
|---|----------|----------|
| Select-a-Size DNA Clean & Concentrator® | 25 preps | D4080    |

### Zymoclean<sup>™</sup> DNA Gel Recovery



DNA fragments recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: individual ladder DNA fragments.

Boost recoveries from agarose gels to > 80%

| Product  | Size                         | Cat. No.       |
|--|------------------------------|----------------|
| Zymoclean™ Gel DNA Recovery Kit (uncapped)             | 50 preps<br>200 preps        | D4001<br>D4002 |
| Zymoclean™ Gel DNA Recovery Kit (capped)               | 50 preps<br>200 preps        | D4007<br>D4008 |
| ZR-96 Zymoclean™ Gel DNA Recovery Kit                  | 2 x 96 preps<br>4 x 96 preps | D4021<br>D4022 |
| Zymoclean <sup>™</sup> Large Fragment DNA Recovery Kit | 25 preps<br>100 preps        | D4045<br>D4046 |

# ZymoBIOMICS® Products



| Product                                    | Cat. No.       | Size               |
|--|----------------|--------------------|
| ZymoBIOMICS® Microbial Community Standards | D6300          | 10 preps           |
| ZymoBIOMICS® DNA Standards                 | D6305<br>D6306 | 200 ng<br>2,000 ng |



| Product  | Cat. No.                         | Size   |
|--|----------------------------------|--|
| DNA/RNA Shield™ Reagent                                      | R1100-50<br>R1100-250            | 50 ml<br>250 ml  |
| DNA/RNA Shield <sup>™</sup> Reagent (2X concentrate)         | R1200-25<br>R1200-125            | 25 ml<br>125 ml  |
| DNA/RNA Shield™ - Lysis Tube (Microbe)                       | R1103                            | 50 pack  |
| DNA/RNA Shield <sup>™</sup> - Lysis Tube (Microbe) with Swab | R1104                            | 50 tubes/50 swabs  |
| DNA/RNA Shield <sup>™</sup> - Swab & Collection Tube         | R1106<br>R1107<br>R1108<br>R1109 | 10 pack (1 ml fill)<br>50 pack (1 ml fill)<br>10 pack (2 ml fill)<br>50 pack (2 ml fill) |
| DNA/RNA Shield™ - Fecal Collection Tube                      | R1101                            | 10 pack  |



| Product                              | Cat. No.       | Size                         |
|--------------------------------------|----------------|------------------------------|
| ZymoBIOMICS® DNA Miniprep Kit        | D4300          | 50 preps                     |
| ZymoBIOMICS® DNA Microprep Kit       | D4301          | 50 preps                     |
| ZymoBIOMICS®-96 Magbead DNA Kit      | D4302          | 2 x 96 preps                 |
| ZymoBIOMICS <sup>®</sup> -96 DNA Kit | D4303<br>D4304 | 2 x 96 preps<br>4 x 96 preps |
| ZymoBIOMICS® RNA Miniprep Kit        | R2001          | 50 preps                     |
| ZymoBIOMICS® DNA/RNA Miniprep Kit    | R2002          | 50 preps                     |



| Product   | Cat. No.       | Size                |
|---|----------------|---------------------|
| ZymoBIOMICS® PCR Premix                             | E2056<br>E2057 | 50 rxns<br>200 rxns |
| Femto <sup>™</sup> Bacterial DNA Quantification Kit | E2006          | 100 rxns            |
| Femto <sup>™</sup> Human DNA Quantification Kit     | E2005          | 100 rxns            |
| Femto <sup>™</sup> Fungal DNA Quantification Kit    | E2007          | 100 rxns            |



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